

DECLARATION  
AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. underneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

## NOVEL POLYMERASE COMPOSITIONS AND USES THEREOF

the specification of which:

is attached hereto  
 was filed in the United States on February 16, 1994 as Application Serial No. 08/197,791.

was filed as PCT international application Serial No. N/A on N/A and was amended under PCT Article 19 on N/A.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119/§172 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119/172
N/A			YES <input type="checkbox"/> NO <input type="checkbox"/>
			YES <input type="checkbox"/> NO <input type="checkbox"/>
			YES <input type="checkbox"/> NO <input type="checkbox"/>
			YES <input type="checkbox"/> NO <input type="checkbox"/>

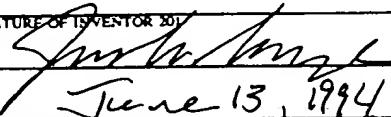
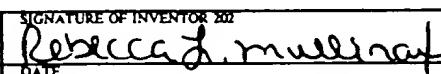
I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED
08/164,290	December 8, 1993		Pending	

POWER OF ATTORNEY: As a named inventor, I hereby appoint S. Leslie Misrock (Reg. No. 18872), Harry C. Jones, III (Reg. No. 20280), Berj A. Terzian (Reg. No. 20060), Gerald J. Flintoft (Reg. No. 20823), David Weild, III (Reg. No. 21094), Jonathan A. Marshall (Reg. No. 24614), Barry D. Rein (Reg. No. 22411), Stanton T. Lawrence, III (Reg. No. 25736), Isaac Jarkovsky (Reg. No. 22713), Joseph V. Colaianni (Reg. No. 20019), Charles E. McKenney (Reg. No. 22795), Philip T. Shannon (Reg. No. 24278), Francis E. Morris (Reg. No. 24615), Charles E. Miller (Reg. No. 24576), Gidon D. Stern (Reg. No. 27469), John J. Lauter, Jr. (Reg. No. 27814), Brian M. Poissant (Reg. No. 28462), Brian D. Coggio (Reg. No. 27624), Rory J. Radding (Reg. No. 28749), Stephen J. Harbulak (Reg. No. 29166), Donald J. Goodell (Reg. No. 19766), James N. Palik (Reg. No. 25510), Thomas E. Friebel (Reg. No. 29258), Laura A. Coruzzi (Reg. No. 30742), Jennifer Gordon (Reg. No. 30753), Jon R. Stark (Reg. No. 30111), Allan A. Fanucci (Reg. No. 30256), Geraldine F. Baldwin (Reg. No. 31232), Victor N. Balancia (Reg. No. 31231), and Albert P. Halluin (Reg. No. 25227), whose address is Pennic & Edmonds, 1155 Avenue of the Americas, New York, New York 10036, and each of them, my attorneys, to prosecute this application, and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO: PENNIE & EDMONDS 1155 AVENUE OF THE AMERICAS NEW YORK, N.Y. 10036-2711			DIRECT TELEPHONE CALLS TO: PENNIE & EDMONDS (212) 790-9090		
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204	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY	ZIP CODE
205	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY	ZIP CODE
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	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY	ZIP CODE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201 	SIGNATURE OF INVENTOR 202 	SIGNATURE OF INVENTOR 203
DATE <u>June 13, 1994</u>	DATE <u>June 24, 1994</u>	DATE
SIGNATURE OF INVENTOR 204	SIGNATURE OF INVENTOR 205	SIGNATURE OF INVENTOR 206
DATE	DATE	DATE

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Sorge et al.

Serial No.: 08/197,791

Group Art Unit: 1814

Filed: February 16, 1994

Examiner: Campbell, E.

For: NOVEL POLYMERASE  
COMPOSITIONS AND USES  
THEREOF

Attorney Docket No.:  
8142-042-999

DECLARATION OF JOSEPH A. SORGE AND REBECCA L. MULLINAX UNDER  
37 CFR § 1.131

Commissioner for Patents and Trademarks  
Washington, D.C. 20231

Sir:

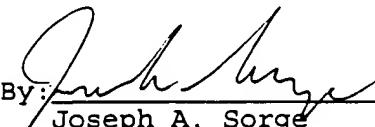
We, JOSEPH A. SORGE and REBECCA L. MULLINAX do declare  
that:

1. We are the co-inventors of the invention described and claimed in the patent application identified above.
2. We reduced to practice the invention claimed in Application Serial No. 08/197,791 prior to February 19, 1993. The reduction to practice took place in the United States.
3. Prior to February 19, 1993, we performed polymerase chain reaction experiments in which the reaction was catalyzed by a mixture of DNA polymerases comprising a DNA polymerase that substantially lacked 5'-3' exonuclease activity and a DNA polymerase that possessed 5'-3' exonuclease activity in order to complement the missing 5'-3' exonuclease activity of the first DNA polymerase.
4. As evidence of the reduction to practice of the invention, we submit copies of pages from the laboratory

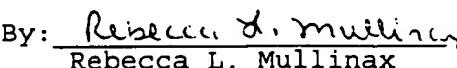
notebook of Rebecca L. Mullinax (Exhibits A and B). These notebook page copies have been modified only to remove references to the actual dates; however, the notebook pages were prepared prior to February 19, 1993.

5. Exhibit A is a copy of a notebook page describing a PCR amplification experiment in which Taq DNA polymerase is to be mixed with Pfu DNA polymerase so as to remove primer mismatches during PCR. The term "Pyrococcus" on this notebook page refers to Pfu DNA polymerase.
6. Exhibit B is a copy of a notebook page describing the results of PCR amplification experiments in which the effectiveness of (i) Pfu DNA polymerase, (ii) Taq DNA polymerase, and (iii) a Taq/Pfu DNA polymerase mixture are compared. The results indicate that significantly more amplification product is obtained with a Taq/Pfu DNA polymerase combination than either Pfu DNA polymerase or Taq DNA polymerase alone. The term "Pyro" on this notebook page refers to Pfu DNA polymerase.
7. We hereby declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Dated: 2/23/96

By:   
Joseph A. Sorge

Dated: 2-23-96

By:   
Rebecca L. Mullinax

Pyrococcus primer design

RecAb

## Purpose

Want to design primers for human 5' 5' HCl. Presence of Ts at 3' end will prime w/ Taq dNTP in template (Kunkel, et al w/ Taq). If a mismatch occurs, Pyrococcus will be able to remove mismatch & extend. Will try polymerization w/ Pyrococcus for several rounds, because taq or use Pyrococcus for all rounds of amplification.

AB-61: AG GTG CAG GTC CTC GAG TCT G G<sup>3'</sup>  
 - - - - - - - - - - - - - - - - - - - - - - T<sup>3'</sup>  
 - - - - - - - - - - - - - - - - - - - - - - T T<sup>3'</sup>  
 - - - - - - - - - - - - - - - - - - - - - - T<sup>3'</sup>  
 - - - - - - - - - - - - - - - - - - - - - - T T<sup>3'</sup>

obtained from Research Genetics

- Joe Sorge's idea

Betty Mullins

D. Mynderse

PCR cont.

RecAb

## 3) results / discussion

results are self-

explanatory

much more PCR prod

when Pyro + Tag are

used in the same mix;

VH splices w/ Seq-in-vitro

as a template have

very little PCR prod

as does CTH when only

Pyro is used; w/ bothTag + Pyro, Fd is

amplified in all splices

(faint w/ AB-713);

previous results w/ Tag

alone demonstrate little.

If any, P Fd amplification

primer seq comparison:

Pyro | Pyro | Pyro + Tag

Tag

|         |     |     |     |     |     |     |     |     |     |     |     |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MC-703: | GTC | CTG | TCC | GAG | GTG | CAG | CTG | CTG | GAG | TCT | GG  |
| AB-714  | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | T   |
| AB-715  | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | TT  |
| AB-716  | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | GGT |
| AB-717  | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | GTT |

diff b/w AB-713 (poor ampl) + other primers is a T instead of a G in the 3' penultimate position  
 in most if not all of the available human Hc seq. The next base after the last G is a G (proline), however, it can be any of the 4 bases; results would indicate that Pyro can remove 1-2 mismatched bases from the 3' end; possibly Pyro is removing the mismatched bases + Tag is extending from the truncated primer template

some VH prod + CTH prod (splices 6-12) w/ Pyro alone, although faint

also used a diff heating/cooling multistep temp profile

D. Mosher